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International application number: PCT/US05/005953

International filing date: 24 February 2005 (24.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 10/785,422
Filing date: 24 February 2004 (24.02.2004)

Date of receipt at the International Bureau: 07 April 2005 (07.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
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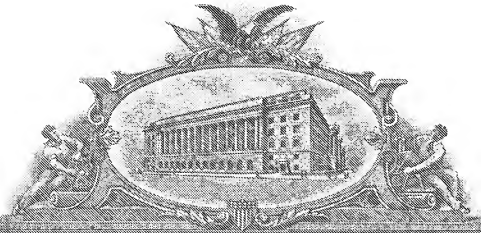

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APPLICATION NUMBER: ***10/785,422***
FILING DATE: ***February 24, 2004***
RELATED PCT APPLICATION NUMBER: ***PCT/US05/05953***

Certified by

Jon W. Dudas

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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	740082.408
First Inventor	Ragina Naidu
Title	SEMI-SYNTHESIS OF TAXANE INTERMEDIATES AND AZIRIDINE ANALOGUES AND THEIR CONVERSION TO PACLITAXEL AND DOCETAXEL
Express Mail Label No.	EV336651752US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☐ Fee Transmittal Form (e.g., PTO/SB/17) *(Submit an original and a duplicate for fee processing)*
2. ☐ Applicant claims small entity status. See 37 CFR 1.27.
3. ☒ Specification [Total Pages 31] *(preferred arrangement set forth below)*
   - Descriptive title of the Invention
   - Cross Reference to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to sequence listing, a table, or a computer program listing appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
4. ☒ Drawing(s) (*35 U.S.C. 113*) [*Total Sheets* 7]
5. Oath or Declaration [*Total Sheets*]
   a. ☐ Newly executed (original or copy)
   b. ☐ Copy from a prior application (37 CFR 1.63 (d)) *(for a continuation/divisional with Box 18 completed)*
      i. ☐ DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☒ Application Data Sheet. See 37 CFR 1.76

ADDRESS TO:

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

7. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program *(Appendix)*
8. ☐ Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. ☐ Computer Readable Form (CRF)
   b. ☐ Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ Paper
   c. ☐ Statements verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

9. ☐ Assignment Papers (cover sheet & document(s))
10. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attorney *(when there is an assignee)*
11. ☐ English Translation Document *(if applicable)*
12. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
13. ☐ Preliminary Amendment
14. ☒ Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. ☐ Nonpublication Request under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or its equivalent.
17. ☐ Other: _____

18. If a CONTINUING APPLICATION OR APPLICATION CLAIMING FOREIGN PRIORITY, *check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:*
☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) ☐ Claims priority from application No.

Prior application information *Examiner* _____ *Group Art Unit:* _____

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

19. CORRESPONDENCE ADDRESS

☐ *Correspondence address below* *or*

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Country			
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Name (Print/Type)	Emily W. Wagner	*Registration No. (Attorney/Agent)*	50,922
Signature	Emily W Wagner	*Date*	February 24, 2004

SEMI-SYNTHESIS OF TAXANE INTERMEDIATES AND AZIRIDINE ANALOGUES AND THEIR CONVERSION TO PACLITAXEL AND DOCETAXEL

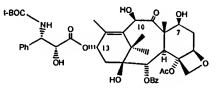
BACKGROUND OF THE INVENTION

Field of the Invention

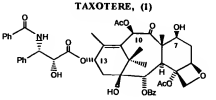
The present invention relates to the semi-synthesis of taxane intermediates and aziridine analogues, in particular, aziridine analogues of cephalomannine and baccatin III intermediates, and their conversion to active antitumor agents, paclitaxel and docetaxel.

Description of the Prior Art

Docetaxel (**1**, Taxotere), a semi-synthetic analog, and paclitaxel (**2**, Taxol), a complex diterpene isolated from the bark of the Pacific yew tree (*Taxus brevifolia*) are arguably the most outstanding cancer chemotherapeutic substances discovered in recent times. While paclitaxel can be obtained from the yew tree or semi-synthetically, only the latter option is currently available for the formation of non-natural docetaxel. The partial synthesis of this important compound has generally been accomplished through esterification of a derivative of the (2*R*, 3*S*) phenylisoserine side chain with a protected form of 10-deacetylbaccatin III, a comparatively abundant natural product also present in the yew tree.



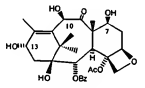
TAXOTERE, (1)



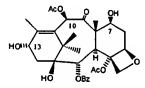
TAXOL, (2)

In Colin's U.S. Pat. No. 4,814,470, it was reported that docetaxel has an activity significantly greater than paclitaxel.

Docetaxel and paclitaxel may be prepared semi-synthetically from 10-deacetylbaccatin III or baccatin III as set forth in U.S. Pat. Nos. 4,924,011 and 4,924,012 or by the reaction of a β-lactam and a suitably protected 10-deacetylbaccatin III or baccatin III derivative as set forth in U.S. Pat. No.5,175,315. 10-deacetylbaccatin III (10-DAB, **3**) and Baccatin III (**4**) can be separated from mixtures extracted from natural sources such as the needles, stems, bark or heartwood of numerous *Taxus* species and have the following structures.

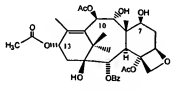


10 DAB, (3)

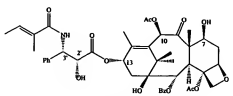


BACC III, (4)

Although, most of the research towards the semi-synthesis of docetaxel and paclitaxel has involved 10-deacetylbaccatin III as the starting material, other taxanes present in the yew tree, such as 9-dihydro-13-acetylbaccatin III (9DHB, **5**), present in the Canadian yew (*Taxus Canadensis*), and cephalomannine (**6**) have been collected and identified.



9DHB, (5)



CEPHALOMANNINE, (6)

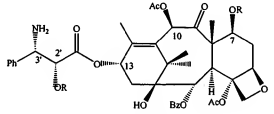
As disclosed in U.S. Pat. Application No. 10/695,416, which application is assigned to the assignee of the present invention, docetaxel and pacliaxel may also be prepared semi-synthetically from 9-dihydro-13-acetylbaccatin III.

Although there have been many advances in the field, there remains a need for new and improved processes for the preparation of taxane intermediates and their conversion to docetaxel and paclitaxel. The present invention addresses these needs and provides further related advantages.

BRIEF SUMMARY OF THE INVENTION

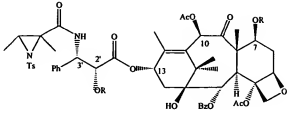
In brief, the present invention relates to the semi-synthesis of novel taxane intermediates and aziridine analogues, in particular, aziridine analogues of cephalomannine and baccatin III intermediates, and their conversion to active antitumor agents, paclitaxel and docetaxel.

In a first embodiment, the present invention provides a process for preparing a taxane comprising the steps of (1) converting cephalomannine to a taxane intermediate having the structure:



wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group, and (2) converting the taxane intermediate to paclitaxel or docetaxel.

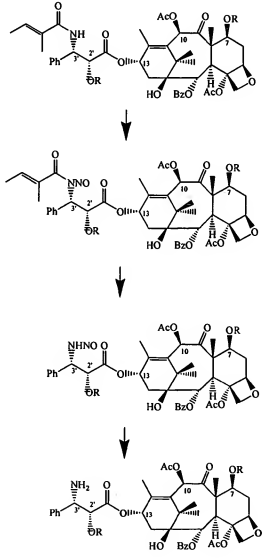
In a more specific embodiment of the foregoing process, the step of converting cephalomannine to the taxane intermediate further comprises the steps of (1) converting cephalomannine to a cephalomannine aziridine analogue having the structure:



wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group, and (2) converting the cephalomannine aziridine analogue to the taxane intermediate.

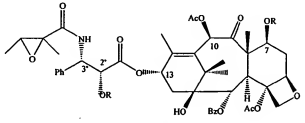
In an alternate more specific embodiment of the foregoing process, the step of converting cephalomannine to the taxane intermediate comprises reacting cephalomannine with formic acid.

In yet another alternate more specific embodiment, the step of converting cephalomannine to the taxane intermediate further comprises the reaction sequence:

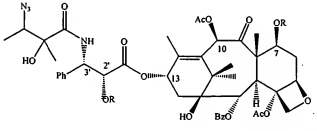


wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group.

In yet another alternate more specific embodiment, the step of converting cephalomannine to the taxane intermediate further comprises the steps of (1) converting cephalomannine to a cephalomannine epoxide analogue having the structure:

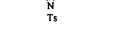


wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group, (2) converting the cephalomannine epoxide analogue to a cephalomannine azido alcohol analogue having the structure:

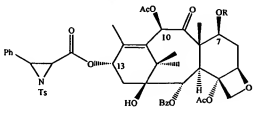


wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group, and (3) converting the cephalomannine azido alcohol analogue to the taxane intermediate.

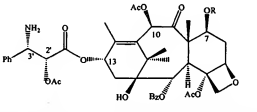
In a second embodiment, the present invention provides a process for preparing a taxane comprising the steps of (1) converting cinnamoyl halide to a cinnamoyl halide aziridine intermediate having the structure:



wherein X is halogen, (2) reacting the cinnamoyl halide aziridine intermediate with protected baccatin III to provide a protected baccatin III aziridine intermediate having the structure:



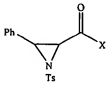
wherein R is selected from hydrogen and a hydroxy-protecting group, (3) converting the protected baccatin III aziridine intermediate to a taxane intermediate having the structure:



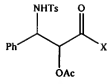
wherein R is selected from hydrogen and a hydroxy-protecting group, and (4) converting the taxane intermediate to paclitaxel or docetaxel.

In a more specific embodiment of the foregoing process X is chloro.

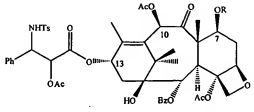
In a third embodiment, the present invention provides a process for preparing a taxane comprising the steps of (1) converting cinnamoyl halide to a cinnamoyl halide aziridine intermediate having the structure:



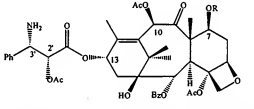
wherein X is halogen, (2) converting the cinnamoyl halide aziridine intermediate to an open chain cinnamoyl halide intermediate having the structure:



wherein X is halogen, (3) reacting the open chain cinnamoyl halide intermediate with protected baccatin III to provide a protected baccatin III intermediate having the structure:

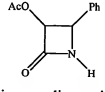


wherein R is selected from hydrogen and a hydroxy-protecting group, (4) converting the protected baccatin III intermediate to a taxane intermediate having the structure:



wherein R is selected from hydrogen and a hydroxy-protecting group, and (5) converting the taxane intermediate to paclitaxel or docetaxel.

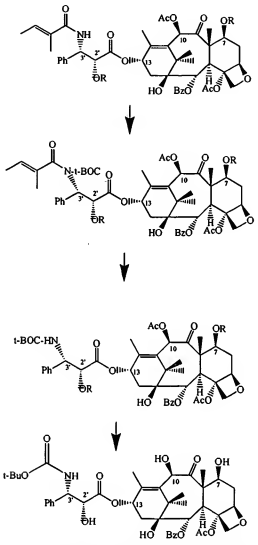
In a more specific embodiment of the foregoing process, the step of reacting the open chain cinnamoyl halide intermediate with protected baccatin III further comprises the steps of (1) converting the open chain cinnamoyl halide intermediate to a β-lactam intermediate having the structure:



and (2) reacting the β-lactam intermediate with protected baccatin III to provide the protected baccatin III intermediate.

In another more specific embodiment of the foregoing process X is chloro.

In a fourth embodiment, the present invention provides a process for preparing docetaxel from cephalomannine comprising the reaction sequence:



wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group.

These and other aspects of the invention will be apparent upon reference to the attached figures and following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1, 2, 3, 4, 5, 6, 7 and 8 illustrate chemical routes for the preparation of taxane intermediates and aziridine analogues, and their conversion to paclitaxel and docetaxel according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

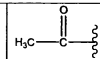
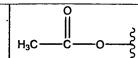
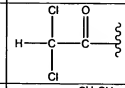
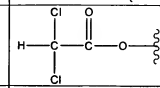
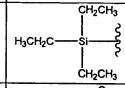
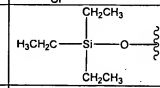
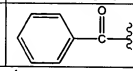
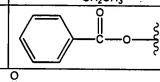
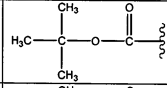
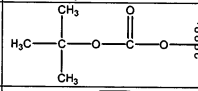
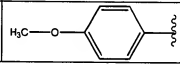
As noted above, the present invention relates to the semi-synthesis of novel taxane intermediates and aziridine analogues, in particular, aziridine analogues of cephalomannine and baccatin III intermediates, and their conversion to active antitumor agents, paclitaxel and docetaxel.

As used herein, the term "hydroxy-protecting group" refers to a readily cleavable group bonded to the oxygen of a hydroxy (-OH) group. Examples of hydroxy protecting groups include, without limitation, acetyl (Ac), benzyl ($PhCH_2$), 1-ethoxyethyl (EE), methoxymethyl (MOM), (methoxyethoxy)methyl (MEM), (p-methoxyphenyl)methoxymethyl (MPM), tert-butyldimethylsilyl (TBS), tert-butyldiphenylsilyl (TBPS), tert-butoxycarbonyl (tBoc, t-Boc, tBOC, t-BOC), tetrahydropyranyl (THP), triphenylmethyl (Trityl, Tr), 2-methoxy-2-methylpropyl, benzyloxycarbonyl (Cbz), trichloroacetyl ($OCCCl_3$), 2,2,2-trichloroethoxycarbonyl (Troc), benzyloxymethyl (BOM), tert-butyl (t-Bu), triethylsilyl (TES), trimethylsilyl (TMS), and triisopropylsilyl (TIPS). The related term "protected hydroxy group" refers to a hydroxy group that is bonded to a hydroxy-protecting group. General examples of protected hydroxy groups include, without limitation, -O-alkyl, -O-acyl, acetal, and -O-ethoxyethyl, where some specific protected hydroxy groups include, formyloxy, acetoxy, propionyloxy, chloroacetoxy, bromoacetoxy, dichloroacetoxy, trichloroacetoxy, trifluoroacetoxy, methoxyacetoxy, phenoxyacetoxy, benzoyloxy, benzoylformoxy, p-nitro benzoyloxy, ethoxycarbonyloxy, methoxycarbonyloxy, propoxycarbonyloxy, 2,2,2-trichloro ethoxycarbonyloxy, benzyloxycarbonyloxy, tert-butoxycarbonyloxy, 1-cyclopropyl ethoxycarbonyloxy, phthaloyloxy, butyryloxy, isobutyryloxy, valeryloxy, isovaleryloxy, oxalyoxy, succinyloxy and pivaloyloxy, phenylacetoxy, phenylpropionyloxy, mesyloxy,

chlorobenzoyloxy, para-nitrobenzoyloxy, para-tert-butyl benzoyloxy, capryloyloxy, acryloyloxy, methylcarbamoyloxy, phenylcarbamoyloxy, naphthylcarbamoyloxy, and the like. Hydroxy-protecting groups and protected hydroxy groups are described in, e.g., C. B. Reese and E. Haslam, "Protective Groups in Organic Chemistry," J. G. W. McOmie, Ed., Plenum Press, New York, N.Y., 1973, Chapters 3 and 4, respectively, and T. W. Greene and P. G. M. Wuts, "Protective Groups in Organic Synthesis," Second Edition, John Wiley and Sons, New York, N.Y., 1991, Chapters 2 and 3.

The following Table shows the chemical structure of some hydroxy-protecting groups, as well as nomenclature used to identify those chemical structures.

TABLE 1

Acetyl (Ac)		Acetoxy (-OAc)	
Dichloroacetyl		Dichloroacetoxy	
Triethylsilyl (TES)		Triethylsiloxy (-OTES)	
Benzoyl		Benzoyloxy	
t-Butyloxycarbonyl (tBOC)			
t-Butoxycarbonyloxy (-O-tBOC)			
para-Methoxyphenyl (PMP)			

The term "alkyl" refers to a hydrocarbon structure wherein the carbons are arranged in a linear, branched, or cyclic manner, including combinations thereof. Lower alkyl refers to alkyl groups of from 1 to 5 carbon atoms. Examples of lower alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, s- and t-butyl and the like. "Cycloalkyl" is a subset of alkyl and includes cyclic hydrocarbon groups of from 3 to 13 carbon atoms. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, norbornyl, adamantyl and the like. When an alkyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be

encompassed; thus, for example, "butyl" is meant to include n-butyl, sec-butyl, isobutyl and t-butyl; propyl includes n-propyl and isopropyl.

The term "alkenyl" refers to an alkyl group having at least one site of unsaturation, *i.e.*, at least one double bond.

The term "alkynyl" refers to an alkyl group having at least one triple bond between adjacent carbon atoms.

The terms "alkoxy" and "alkoxyl" both refer to moieties of the formula -O-alkyl. Examples include methoxy, ethoxy, propoxy, isopropoxy, cyclopropyloxy, cyclohexyloxy and the like. Lower-alkoxy refers to groups containing one to four carbons. The analogous term "aryloxy" refers to moieties of the formula –O-aryl.

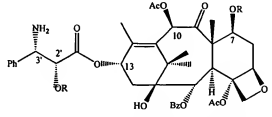
The term "acyl" refers to moieties of the formula –C(=O)-alkyl. One or more carbons in the acyl residue may be replaced by nitrogen, oxygen or sulfur as long as the point of attachment to the parent remains at the carbonyl. Examples include acetyl, benzoyl, propionyl, isobutyryl, t-butoxycarbonyl, benzyloxycarbonyl and the like. Lower-acyl refers to groups containing one to four carbons.

The term "aryl" refers to phenyl or naphthyl. Substituted aryl refers to mono- and poly- substituted phenyl or naphthyl. Exemplary substituents for aryl include one or more of halogen, hydroxyl, alkoxy, aryloxy, heteroaryloxy, amino, alkylamino, dialkylamino, mercapto, alkylthio, arylthio, heteroarylthio, cyano, carboxyl, alkoxycarbonyl where the alkoxy portion contains 1 to 15 carbons, aryloxycarbonyl where the aryloxy portion contains 6 to 20 carbon, or heteroarylcarbonyl where the heteroaryl portion contains 3 to 15 carbon atoms.

The term "heteroaryl" refers to a 5- or 6-membered heteroaromatic ring containing 1-3 heteroatoms selected from O, N, or S; a bicyclic 9- or 10-membered heteroaromatic ring system containing 0-3 heteroatoms selected from O, N, or S; or a tricyclic 13- or 14-membered heteroaromatic ring system containing 0-3 heteroatoms selected from O, N, or S. Exemplary aromatic heterocyclic rings include, e.g., imidazole, pyridine, indole, thiophene, benzopyranone, thiazole, furan, benzimidazole, quinoline, isoquinoline, quinoxaline, pyrimidine, pyrazine, tetrazole and pyrazole.

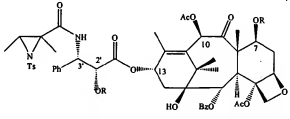
The term "halogen" refers to fluoro, chloro, bromo or iodo.

In a first embodiment, the present invention provides a process for preparing a taxane comprising the steps of (1) converting cephalomannine to a primary amine taxane intermediate having the structure:



wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group, and (2) converting the taxane intermediate to paclitaxel or docetaxel.

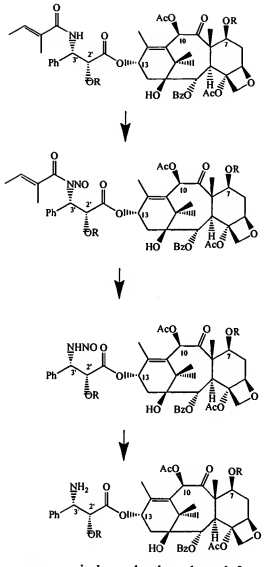
In a more specific embodiment, cephalomannine is converted to a cephalomannine aziridine analogue having the structure:



wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group, by substituting the double bond of the C-13 side chain of cephalomannine with an aziridine ring. The cephalomannine aziridine analogue is subsequently hydrolyzed to give the primary amine taxane intermediate.

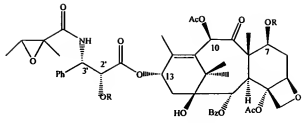
In an alternate more specific embodiment, cephalomannine is directly hydrolyzed with formic acid to give the primary amine taxane intermediate.

In yet another alternate more specific embodiment, cephalomannine is converted to the primary amine taxane intermdiate by nitrosation using sodium nitrite in $AcOH:Ac_2O$ or N_2O_4 in acetonitrile, followed by lithium hydroxide and 30% hydrogen peroxide hydrolysis and, then, Raney-Nickel reduction according to the reaction sequence:

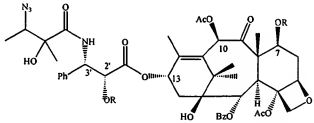


wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group.

In yet another alternate more specific embodiment, cephalomannine is converted to a cephalomannine epoxide analogue having the structure:

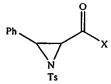


wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group, which is then reacted with sodium azide in methanol at 65°C to give a cephalomannine azido alcohol analogue having the structure:

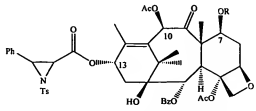


wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group, which is then reduced to the give the primary amine taxane intermediate.

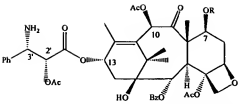
In a second embodiment, the present invention provides a process for preparing a taxane comprising the steps of (1) converting cinnamoyl halide to a cinnamoyl halide aziridine intermediate having the structure:



wherein X is halogen, (2) coupling the cinnamoyl halide aziridine intermediate with protected baccatin III using NaH, DCM to provide a protected baccatin III aziridine intermediate having the structure:



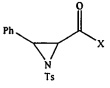
wherein R is selected from hydrogen and a hydroxy-protecting group, (3) hydrolyzing the protected baccatin III aziridine intermediate to a taxane intermediate having the structure:



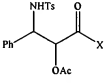
wherein R is selected from hydrogen and a hydroxy-protecting group, and (4) converting the taxane intermediate to paclitaxel or docetaxel.

In a more specific embodiment of the foregoing process, X is chloro.

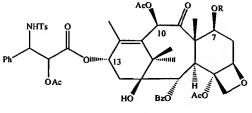
In a third embodiment, the present invention provides a process for preparing a taxane comprising the steps of (1) converting cinnamoyl halide to a cinnamoyl halide aziridine intermediate having the structure:



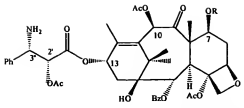
wherein X is halogen, (2) reacting the cinnamoyl halide aziridine intermediate with acetic acid to give an open chain cinnamoyl halide intermediate having the structure:



wherein X is halogen, (3) coupling the open chain cinnamoyl halide intermediate with protected baccatin III using NaH, DCM to provide a protected baccatin III intermediate having the structure:

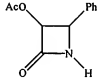


wherein R is selected from hydrogen and a hydroxy-protecting group, (4) hydrolyzing the protected baccatin III intermediate to a taxane intermediate having the structure:



wherein R is selected from hydrogen and a hydroxy-protecting group, and (5) converting the taxane intermediate to paclitaxel or docetaxel.

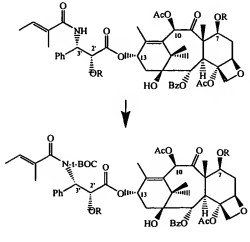
In a more specific embodiment of the foregoing process, the step of reacting the open chain cinnamoyl halide intermediate with protected baccatin III further comprises the steps of (1) converting the open chain cinnamoyl halide intermediate to a β-lactam intermediate having the structure:

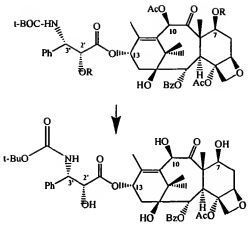


and (2) reacting the β-lactam intermediate with protected baccatin III to provide the protected baccatin III intermediate.

In another more specific embodiment of the foregoing process, X is chloro.

In a fourth embodiment, the present invention provides a process for preparing docetaxel from cephalomannine by introduction of a t-BOC group at the secondary amine of protected cephalomannine followed by hydrolysis with lithium hydroxide in THF, and deprotection at the 2', 7 and 10 positions according to the reaction sequence:





wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group.

EXAMPLES

The following Examples disclose specific processes for synthesizing various aziridine analogues, and their conversion to paclitaxel and docetaxel. The disclosed processes may be utilized with both purified and partially purified taxanes. Unless otherwise noted, all scientific and technical terms have the meanings as understood by one of ordinary skill in the art.

Example 1

Aziridination of cephalomannine

As shown in Figure 1, cephalomannine (0.12 mmol) was dissolved in dry freshly distilled acetonitrile (1 ml) at room temperature under anhydrous conditions. To this solution was added chloroamine-T (0.18 mmol), followed by copper triflate (0.12 mmol) with vigorous stirring. The mixture was stirred under slightly warming (25 °C) conditions until all starting material were consumed. The mixture was worked up and

purified by column chromatography using mixtures of dichloromethane and ethyl acetate to give white crystals of the cephalomannine aziridine analogue.

Preparation of primary amine taxane intermediate

Process 1. To a solution of the above cephalomannine aziridine analogue (0.025 mmol) in dry benzene (5 ml) were added *o*-phenylenediamine (0.025 mmol) and *p*-toluenesulfonic acid (catalytic, 2 mg). The mixture was refluxed for 16 h until all starting material was consumed (TLC). The mixture was allowed to cool to room temperature, diluted with ethyl acetate and washed successively with dilute HCl (1N) followed by water and brine. The organic layer was dried and purified by column chromatography using mixtures of dichloromethane and ethyl acetate to yield the primary amine taxane intermediate.

Process 2. To a 0.2 M solution of the above cephalomannine aziridine analogue (3.51 mmol) in tetrahydrofuran was added 10.54 ml (10.54 mmol) of a 1.0 N solution of lithium hydroxide. The solution was stirred for 12 h at room temperature. After removal of tetrahydrofuran in vacuo, the basic aqueous residue was acidified by the addition of 10% acetic acid and extracted with ether. Drying ($MgSO_4$) and concentration afforded the crude material that was purified by column chromatography to afford the pure white solid of the primary amine taxane intermediate. (Note: The following could also be used: 10 equiv. LiOH, 20 equiv. 30% H_2O_2, 3:1 THF:H_2O, time, 0⇒T °C; Na_2SO_3, 5 min. 0 °C).

Conversion of primary amine taxane intermediate to paclitaxel or docetaxel

A sample of the primary amine taxane intermediate (0.091 mmol) was dissolved in ethyl acetate (9.1 ml) and a saturated solution of $NaHCO_3$ (9.1 ml) was added. To this biphasic mixture was added di-tert-butyl dicarbonate (0.18 mmol). The mixture was stirred for 12 h at room temperature and TLC showed complete consumption of the starting material. The reaction was worked up as usual and the residue purified by column chromatography using mixtures of dichloromethane and ethyl acetate or acetone to give

docetaxel. The ^{1}H NMR, ^{13}C NMR and mass spectra data for the isolated material match with the reported data for docetaxel.

To convert the primary amine to taxol, there are several methods that could be used, such as the method disclosed in U.S. Patent No. 5,808,113, which is incorporated herein by reference in its entirety.

Example 2

Hydrolysis of cephalomannine

As shown in Figure 2, cephalomannine was dissolved in formic acid at 0 °C, stirred at this temperature for 12 h, poured over crushed ice and worked up as usual. The crude residue was purified by column chromatography using mixtures of dichloromethane and ethyl acetate to afford the pure primary amine taxane intermediate.

Example 3

Aziridination of cinnamoyl chloride

As shown in Figure 3, to a mixture of cinnamoyl chloride and anhydrous chloramine-T in acetonitrile was added phenyltrimethylammonium tribromide (PTAB) at room temperature. After 12 h of vigorous stirring, the reaction mixture was concentrated and filtered through a short column of silica gel and eluted with 10% ethyl acetate in hexanes. After evaporation of the solvent, the resultant solid was purified by column chromatography or recrystallization to afford the cinnamoyl chloride aziridine intermediate.

Acid-catalyzed ring opening

As further shown in Figure 3, the cinnamoyl chloride aziridine intermediate was dissolved in aqueous acetic acid at 0 °C, stirred at this temperature for 10 h and worked up as usual. Purification of the crude mixture by column chromatography and crystallization afforded the open chain cinnamoyl chloride intermediate.

Preparation of β-lactam intermediate

As shown in Figure 4, the above open chain cinnamoyl chloride intermediate was cyclized to form the β-lactam intermediate using methods well known in the literature.

Example 4

Coupling Reaction

As shown in Figure 5, the open chain cinnamoyl chloride intermediate and C7 protected baccatin III were dissolved in anhydrous freshly distilled THF under argon atmosphere at room temperature. The stirred solution was cooled to 0 °C and added to a suspension of NaH in THF at 0 °C. The solution was warmed slowly to room temperature and maintained at this temperature for 3 h. The reaction mixture was cooled to 0 °C and quenched with brine. The reaction mixture was extracted with dichloromethane and the combined extracts were washed several times with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography using mixtures of hexanes and ethyl acetate to afford the pure coupled protected baccatin III intermediate that could be hydrolyzed to give the primary amine taxane intermediate. Although this reaction is illustrated in Figure 5 with sodium hydride, in other embodiments of the present invention the coupling may be performed in the presences of a metal alkoxide, *e.g.*, sodium hexamethyldisalide or lewis acid.

Example 5

Nitrosation

As shown in Figure 6, to a solution of cephalomannine (0.76 mmol) in glacial acetic acid (2.5 ml) and acetic anhydride (5 ml) at 0 °C was added $NaNO_2$ (7.6 mmol). The resulting solution was stirred under argon at 0 °C for 16 h and then poured over ice and extracted with diethyl ether. The combined organic extracts were washed with

water, 5% Na_2CO_3, water and saturated NaCl and dried over $MgSO_4$. The dry extracts were filtered and then concentrated in vacuo, and the crude product was purified by column chromatography using mixtures of hexane-ethyl acetate to afford the pure product.

Hydrolysis

To the above solution in tetrahydrofuran was added a 1.0 N solution of lithium hydroxide. The solution was stirred for 12 h at room temperature. After removal of tetrahydrofuran in vacuo, the basic aqueous residue was acidified by the addition of 10% acetic acid and extracted with ether. Drying ($MgSO_4$) and concentration afforded the crude material that was purified by column chromatography to afford the pure white solid of the primary amine taxane intermediate. (Note: The following could also be used: 10 equiv. LiOH, 20 equiv. 30% H_2O_2, 3:1 THF:H_2O, time, 0⇒T °C; Na_2SO_3, 5 min. 0 °C).

Reduction

The above hydrolyzed product was dissolved in ethanol at room temperature and Raney-Nickel was added in one portion to the stirred solution. The reaction mixture was stirred at this temperature and treated with hydrogen, until the complete consumption of the starting material. The reaction mixture was filtered and the filtrate evaporated. The residue was dissolved in an inert solvent such as dichloromethane and worked up as usual. The crude product was purified by column chromatography using mixtures of dichloromethane and ethyl acetate to afford the pure product.

Example 6

Preparation of N-acyl derivative

As shown in Figure 7, to a solution of cephalomannine (9.47 mmol) in dichloromethane was added triethylamine (9.47 mmol), di-tert-butyl dicarbonate (18.94 mmol), and 4-(dimethylamino)pyridine (DMAP) (9.47 mmol). The solution was stirred for 12 h at room temperature under an argon atmosphere. The volatiles were removed and the

residue was purified by column chromatography. Elution with dichloromethane and ethyl acetate afforded the cephalomannine N-t-BOC derivative.

Alternatively, DMAP (0.1 mmol) was added to a stirred solution of the cephalomannine (1.0 mmol) in dry acetonitrile followed by BOC_2O (1.1 mmol). After stirring for 10 h at room temperature, all starting material was consumed (TLC). The reaction mixture was evaporated at room temperature and the residue partitioned between ether and aqueous $KHSO_4$. The organic extract was thoroughly washed in turn with aqueous solution of $KHSO_4$ and $NaHCO_3$ and finally brine and dried over $MgSO_4$. Evaporation to complete dryness left a light yellow residue that was purified by column chromatography to afford the cepahlomannine N-t-BOC derivative.

Example 7

Preparation of cephalomannine epoxide analogue

As shown in Figure 8, to a solution of cephalomannine in dichloromethane was added $NaHCO_3$ followed by MCPBA at -15 °C. The reaction was worked up as usual after the consumption of the starting material and purified by column chromatography using mixtures of dichloromethane and ethyl acetate to afford the pure cephalomannine epoxide analogue.

Preparation of cephalomannnine azido alcohol analogue

The cephalomannine epoxide analogue was dissolved in methanol and aqueous solution of NaN_3 was added at room temperature. The solution was heated to 65 °C for 12 h. The reaction mixture was cooled to room temperature and worked up as usual and purified by column chromatography using mixtures of dichloromethane and ethyl acetate to afford the pure cephalomannine azido alcohol analogue.

All of the above U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications

referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference, in their entirety.

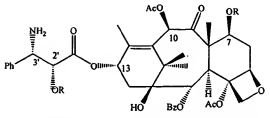
From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is claimed is:

1. A process for preparing a taxane comprising the steps of:

converting cephalomannine to a taxane intermediate having the structure:



wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group; and

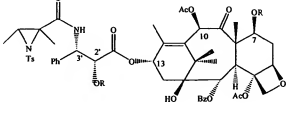
converting the taxane intermediate to paclitaxel or docetaxel.

2. The process of claim 1 wherein the taxane intermediate is converted to paclitaxel.

3. The process of claim 1 wherein the taxane intermediate is converted to docetaxel.

4. The process of claim 1 wherein the step of converting cephalomannine to the taxane intermediate further comprises the steps of:

converting cephalomannine to a cephalomannine aziridine analogue having the structure:

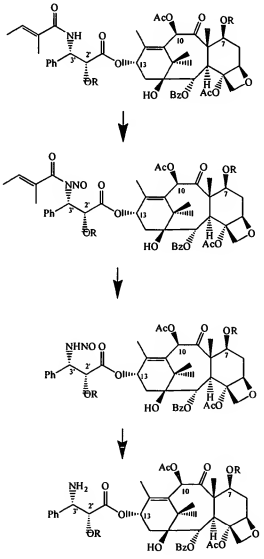


wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group; and

converting the cephalomannine aziridine analogue to the taxane intermediate.

5. The process of claim 1 wherein the step of converting cephalomannine to the taxane intermediate comprises reacting cephalomannine with formic acid.

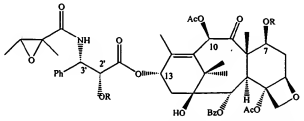
6. The process of claim 1 wherein the step of converting cephalomannine to the taxane intermediate further comprises the reaction sequence:



wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group.

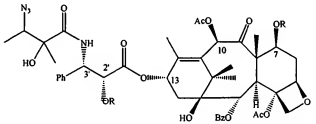
7. The process of claim 1 wherein the step of converting cephalomannine to the taxane intermediate further comprises the steps of:

converting cephalomannine to a cephalomannine epoxide analogue having the structure:



wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group;

converting the cephalomannine epoxide analogue to a cephalomannine azido alcohol analogue having the structure:

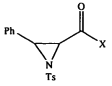


wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group; and

converting the cephalomannine azido alcohol analogue to the taxane intermediate.

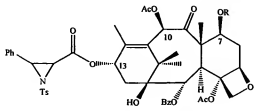
8. A process for preparing a taxane comprising the steps of:

converting cinnamoyl halide to a cinnamoyl halide aziridine intermediate having the structure:



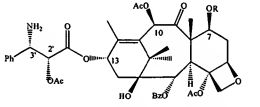
wherein X is halogen;

reacting the cinnamoyl halide aziridine intermediate with protected baccatin III to provide a protected baccatin III aziridine intermediate having the structure:



wherein R is selected from hydrogen and a hydroxy-protecting group;

converting the protected baccatin III aziridine intermediate to a taxane intermediate having the structure:



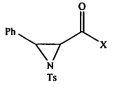
wherein R is selected from hydrogen and a hydroxy-protecting group; and

converting the taxane intermediate to paclitaxel or docetaxel.

9. The process of claim 8, wherein X is chloro.

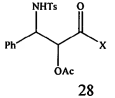
10. A process for preparing a taxane comprising the steps of:

converting cinnamoyl halide to a cinnamoyl halide aziridine intermediate having the structure:



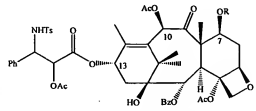
wherein X is halogen;

converting the cinnamoyl halide aziridine intermediate to an open chain cinnamoyl halide intermediate having the structure:



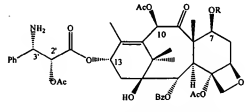
wherein X is halogen;

reacting the open chain cinnamoyl halide intermediate with protected baccatin III to provide a protected baccatin III intermediate having the structure:



wherein R is selected from hydrogen and a hydroxy-protecting group;

converting the protected baccatin III intermediate to a taxane intermediate having the structure:



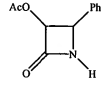
wherein R is selected from hydrogen and a hydroxy-protecting group; and

converting the taxane intermediate to paclitaxel or docetaxel.

11. The process of claim 10, wherein X is chloro.

12. The process of claim 10, wherein the step of reacting the open chain cinnamoyl halide intermediate with protected baccatin III further comprises the steps of:

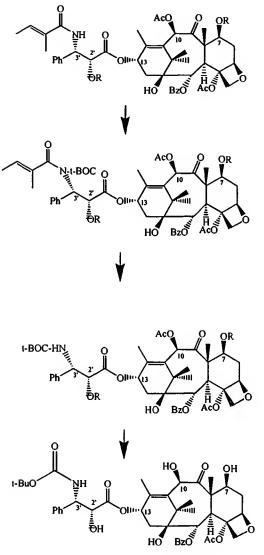
converting the open chain cinnamoyl halide intermediate to a β-lactam intermediate having the structure:



; and

reacting the β-lactam intermediate with protected baccatin III to provide the protected baccatin III intermediate.

13. A process for preparing docetaxel from cephalomannine comprising the reaction sequence:



wherein R is at each occurrence independently selected from hydrogen and a hydroxy-protecting group.

ABSTRACT

A process is provided for the semi-synthesis of taxane intermediates and aziridine analogues of cephalomannne and baccatin III intermediates, and the conversion of such intermediates and analogues to paclitaxel and docetaxel.

458926_1.DOC

FIGURE 1

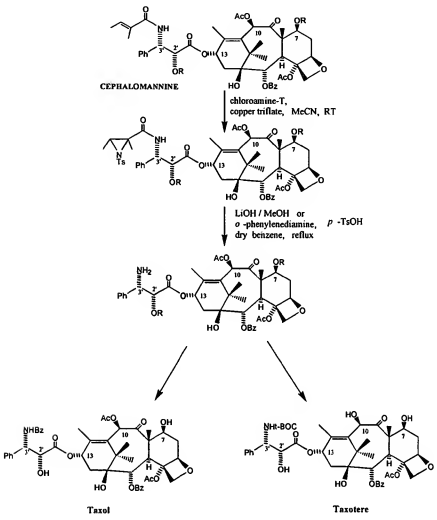

CEPHALOMANNINE
chloroamine-T,
copper triflate, MeCN, RT
LiOH / MeOH or
o -phenylenediamine,
dry benzene, reflux
p -TsOH
Taxol
Taxotere

FIGURE 2

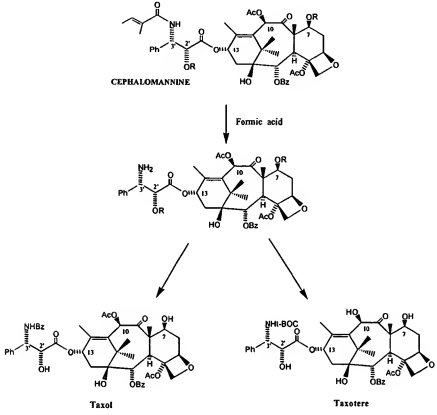


FIGURE 3

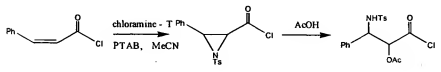


FIGURE 4

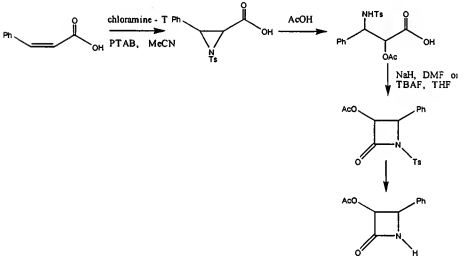


FIGURE 5

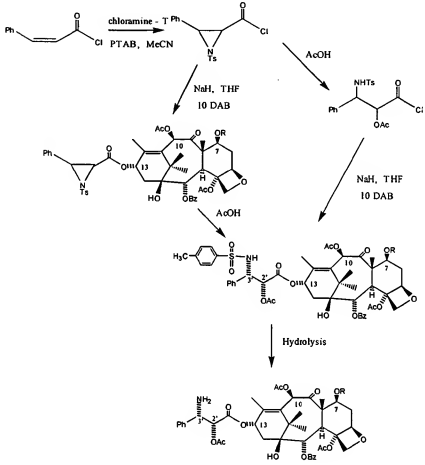

chloramine - T
PTAB, MeCN
AcOH
NaH, THF
10 DAB
NaH, THF
10 DAB
AcOH
Hydrolysis
Primary amine

FIGURE 6

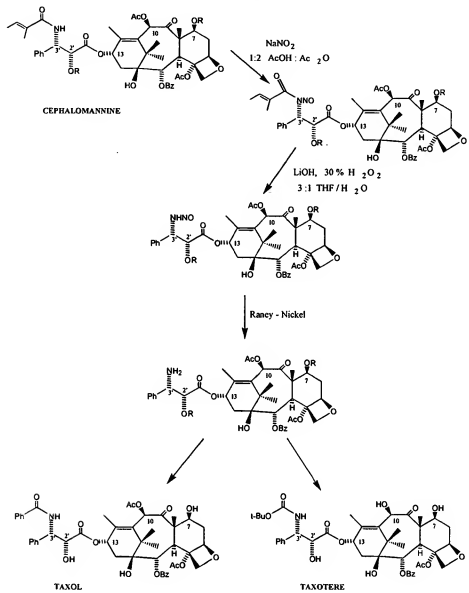

CEPHALOMANNINE
NaNO2
1:2 AcOH : Ac 2O
LiOH, 30 % H 2O2
3 :1 THF / H 2O
Raney - Nickel
TAXOL
TAXOTERE

FIGURE 7

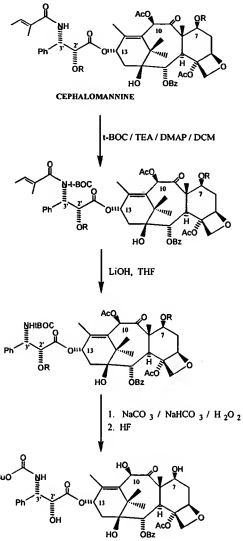
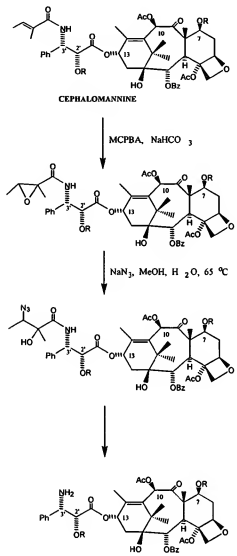

CEPHALOMANNINE
t-BOC / TEA / DMAP / DCM
LiOH, THF
1. NaCO 3 / NaHCO 3 / H 2O 2
2. HF
TAXOTERE

FIGURE 8



APPLICATION DATA SHEET

Application Information

Application number::	
Filing Date::	
Application Type::	Regular
Subject Matter::	Utility
Suggested classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	None
Number of CD disks::	
Number of copies of CDs::	
Sequence submission?::	
Computer Readable Form (CRF)?::	No
Number of copies of CRF::	
Title ::	SEMI-SYNTHESIS OF TAXANE INTERMEDIATES AND AZIRIDINE ANALOGUES AND THEIR CONVERSION TO PACLITAXEL AND DOCETAXEL
Attorney Docket Number::	740082.408
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	
Total Drawing Sheets::	7
Small Entity?::	Yes
Petition included?::	No
Petition Type::	
Licensed U.S. Gov't Agency::	
Contract or Grant No::	
Secrecy Order in Parent Appl.?::	No

First Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	Canada
Status::	Full Capacity
Given Name::	Ragina
Middle Name::	
Family Name::	Naidu
Name Suffix::	
City of Residence::	Burnaby
State or Province of Residence::	BC
Country of Residence::	Canada
Street of mailing address::	3768 Brandon Street
City of mailing address::	Burnaby
State or Province of mailing address::	BC
Country of mailing address::	Canada
Postal or Zip Code of mailing address::	V5G 2P2

Correspondence Information

Correspondence Customer Number :: **00500**

Representative Information

Representative Customer Number::		**00500**

Domestic Priority Information

Application ::	Continuity Type::	Parent Application::	Parent Filing Date::

Foreign Priority Information

Country::	Application number::	Filing Date::	Priority Claimed::

Assignee Information

Assignee name::	Phytogen Life Sciences Inc.
Street of mailing address::	1527 Cliveden Avenue
City of mailing address::	Delta
State or Province of mailing address::	BC
Country of mailing address::	Canada
Postal or Zip Code of mailing address::	V3M 6P7